F7 Cas9-CKO Strategy

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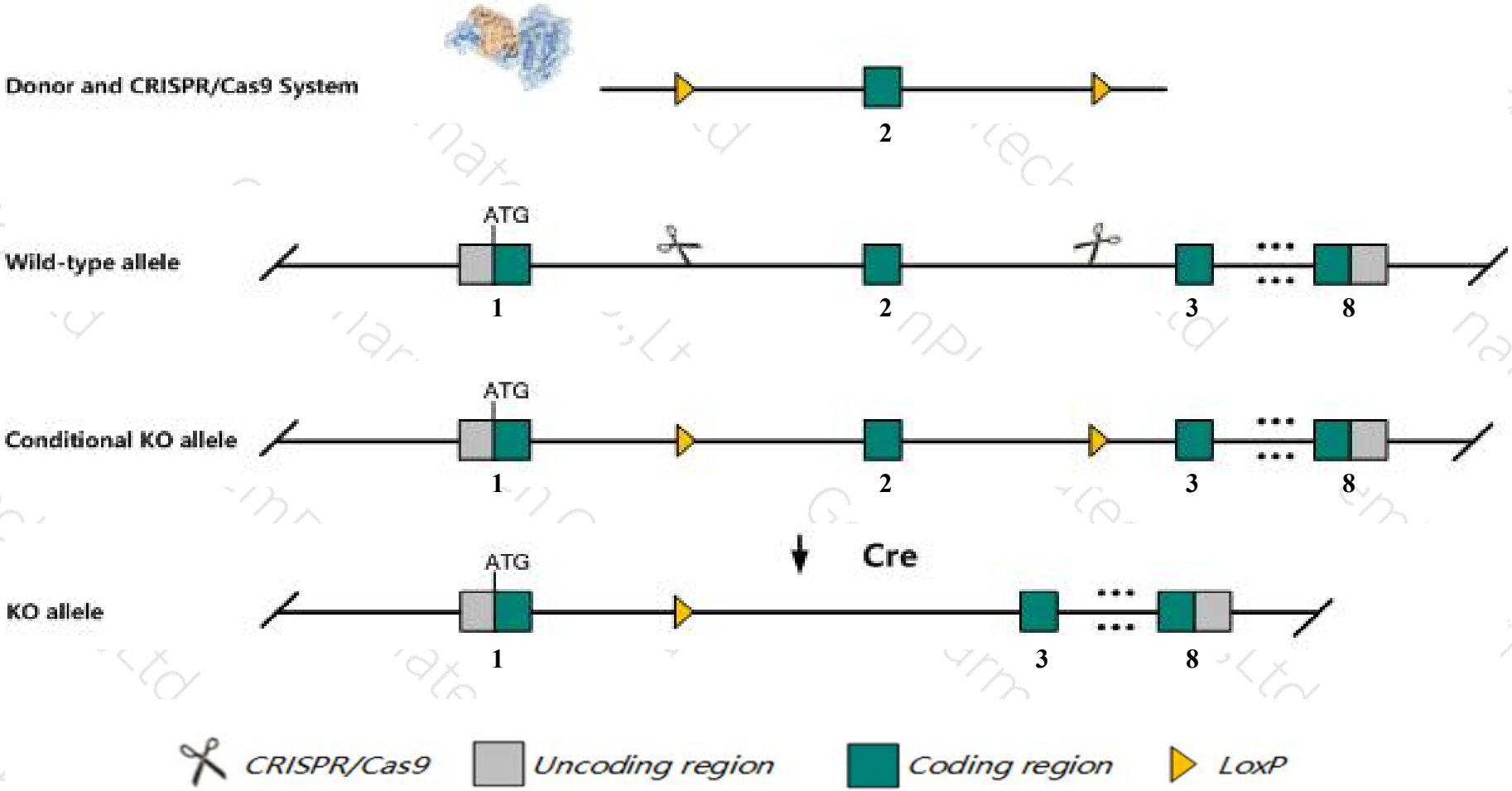
Project Overview

Project Name	***F7***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *F7* gene. The schematic diagram is as follows:



Technical routes

- The *F7* gene has 1 transcript. According to the structure of *F7* gene, exon2 of *F7-201* (ENSMUST00000033820.3) transcript is recommended as the knockout region.The region contains 161bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *F7* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

- According to the existing MGI data,Mice homozygous for a targeted null mutation developed normally through embryogenesis, and exhibited no vascular defects; however, 70% of homozygous neonates suffered fatal intra-abdominal haemorrhaging and died within 24 hours after birth.

- The *F7* gene is located on the Chr8. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

- This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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F7 coagulation factor VII [*Mus musculus* (house mouse)]

Gene ID: 14068, updated on 12-Aug-2019

Summary

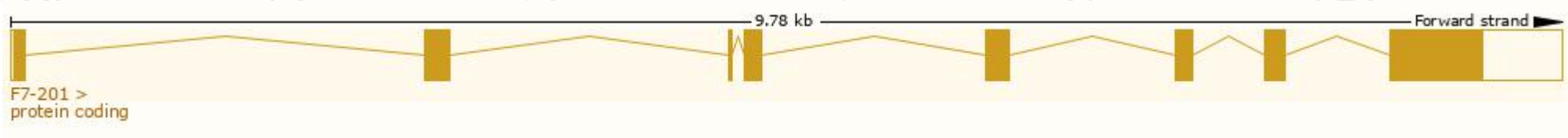
Official Symbol	F7 provided by MGI
Official Full Name	coagulation factor VII provided by MGI
Primary source	MGI:MGI:109325
See related	Ensembl:ENSMUSG00000031443
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cf7; FVII; AI132620
Summary	This gene encodes a vitamin K-dependent serine protease that plays a critical role in the extrinsic pathway of blood coagulation. Upon contact with tissue factor III (TF III), the encoded protein forms an activated complex termed TF-FVIIa that initiates the coagulation cascade involving other coagulation factors, ultimately resulting in a fibrin clot. Complete lack of the encoded protein in mice results in in perinatal lethality due to bleeding from normal blood vessels. [provided by RefSeq, Apr 2015]
Expression	Biased expression in liver adult (RPKM 49.6), liver E18 (RPKM 7.5) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

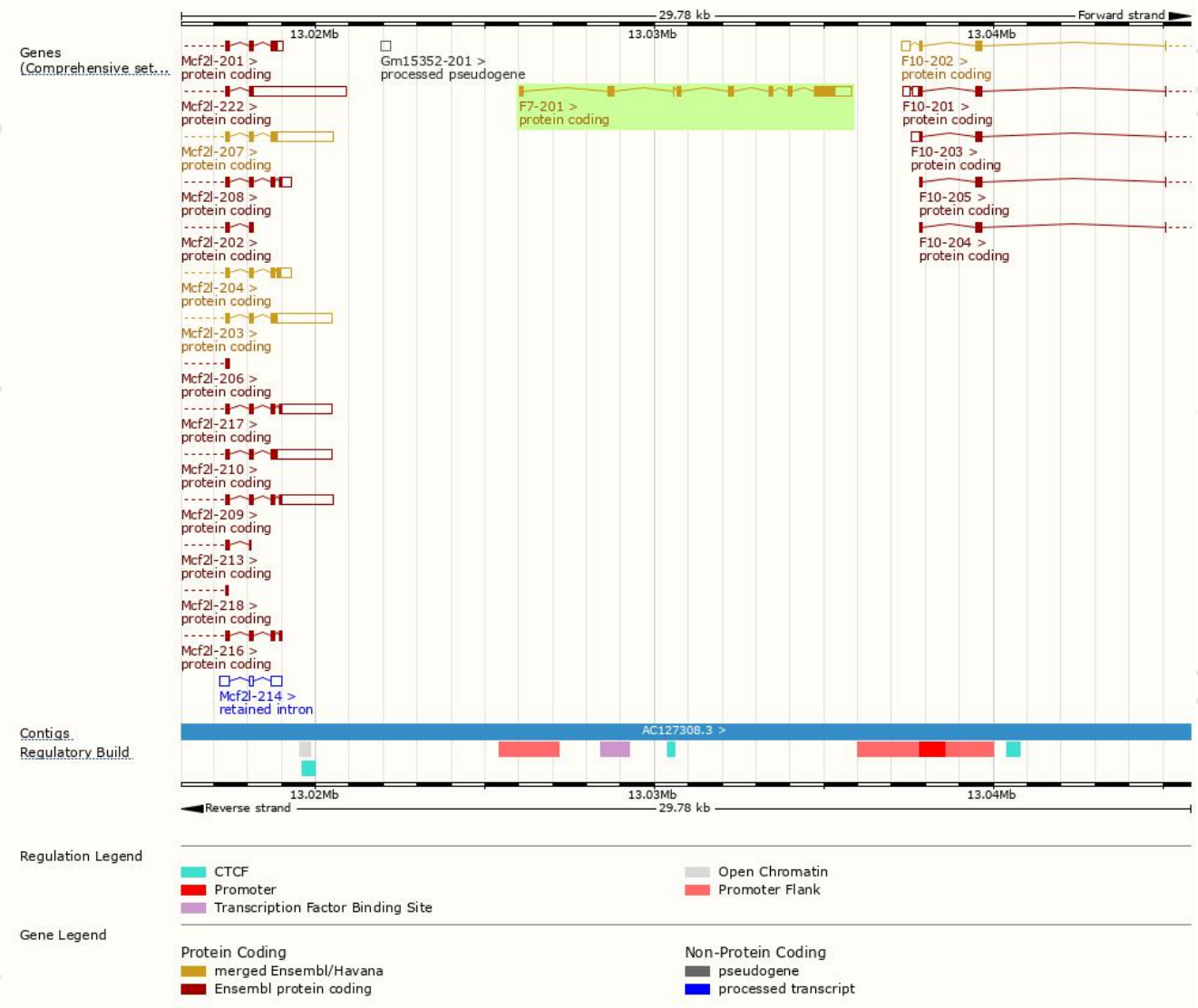
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
F7-201	ENSMUST00000033820.3	1859	446aa	Protein coding	CCDS22104	P70375 Q542C2	TSL:1 GENCODE basic APPRIS P1

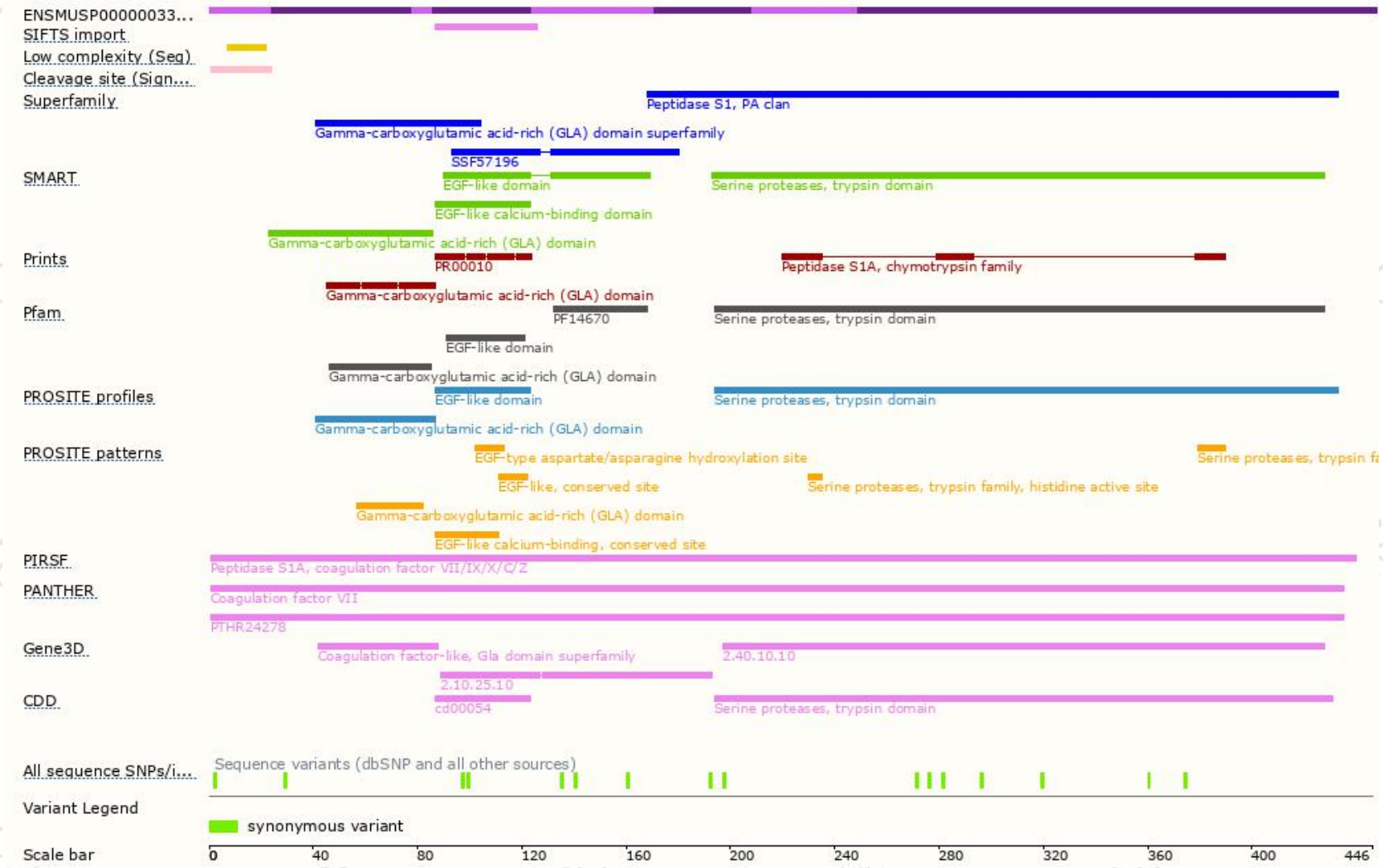
The strategy is based on the design of *F7-201* transcript,The transcription is shown below



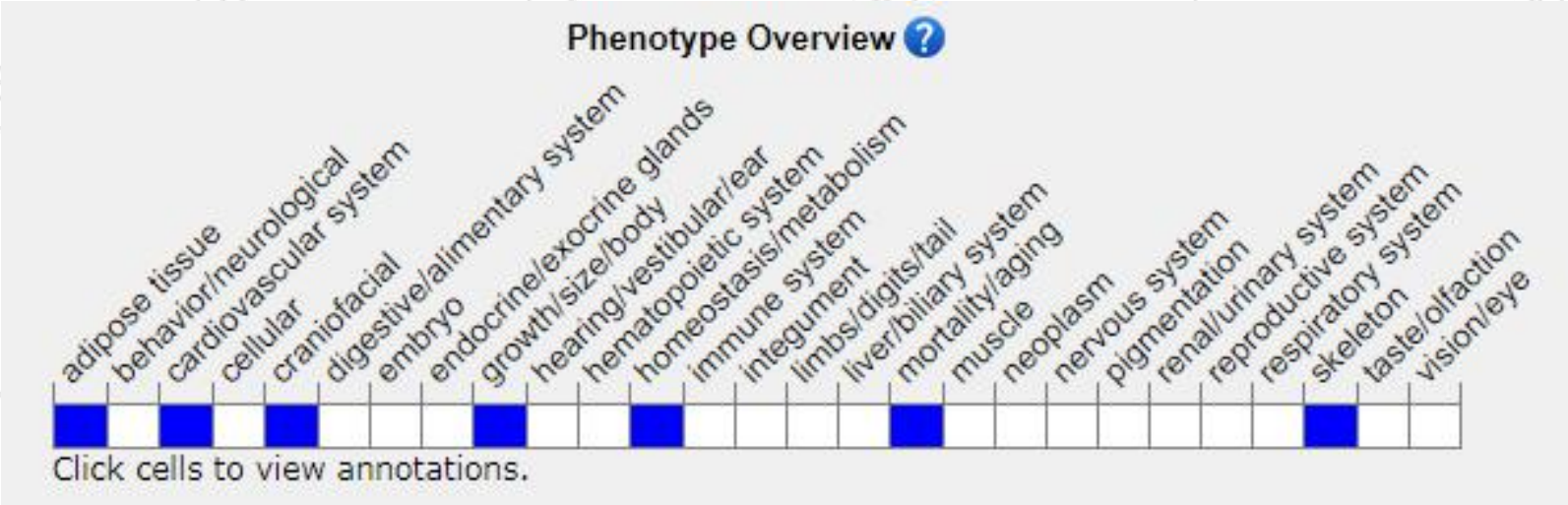
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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